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Phenotypic characterization of macrophages in human term placenta

B. MUES,* D. LANGER,† G. ZWADLO* & C. SORG* **Institute of Experimental Dermatology and* †*Clinic for Obstetrics and Gynecology, University of Münster, Münster, FRG*

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SUMMARY

Immunohistological techniques have been used to study heterogeneity, frequency and distribution of macrophages and T lymphocytes in chorionic villous mesenchyme, stroma of the amniochorion and decidua of 36 human term placentas obtained at spontaneous normal delivery and by caesarean section, using a panel of monoclonal antibodies (mAb) specific for macrophage phenotypes appearing in acute early (mAb 27E10), late (mAb 25F9) and down-regulatory (mAb RM3/1) stages of inflammation. Significant numbers of macrophages were identified. It could be shown that RM3/1$^+$ macrophage phenotypes which *in vitro* are strongly dexamethasone-inducible and *in vivo* appear in down-regulatory stages of inflammatory processes are the major cell population in human term placenta. Macrophages characterized by monoclonal antibodies 27E10 and 25F9, as well as CD4$^+$ and CD8$^+$ cells, were distributed sparsely or were completely absent. The finding of anti-inflammatory macrophage phenotypes to be the predominant mononuclear cell population in human term placenta provides support for a mechanism whereby placenta functions as an active immunosuppressive biological barrier between mother and fetus.

INTRODUCTION

Placenta is an immunologically privileged organ, which plays a major role in protecting the fetus against maternal infections and anti-fetal immune reactions. Many different immune mechanisms have been proposed to account for the fact that the fetal allograft is not rejected by the mother (Jacoby, Olding & Oldstone, 1984; Lala *et al.*, 1983; Billingham, 1981). There is some evidence that the fetus is protected from the mother's immune system by active immune suppression mediated by antigen-specific maternal CD8$^+$ cells which inhibit the generation of cytotoxic T lymphocytes to fetal antigens (Thomas & Erickson, 1986; Chaouat & Voisin, 1979). However, data have been conflicting concerning the role of T lymphocytes in placenta during pregnancy (Engleman *et al.*, 1972; Bulmer & Sunderland, 1984). On the other hand, substantial numbers of macrophages, which are supposed to play a major role within placental immune system, can be found in the chorionic villous mesenchyme and decidua of placenta throughout pregnancy (Bulmer & Johnson, 1984; Castellucci, Zaccheo & Pescetto, 1980). In particular, the Hofbauer cells have been identified as mononuclear phagocytes by properties like their cell surface receptors and ultrastructural and histochemical characteristics (Fox & Kharkongor, 1969; Enders & King, 1970). Placental mononuclear phagocytes express receptors for Fc (IgG) and C3, bear a cytophilic surface immunoglobulin and actively participate in both immune and non-immune phagocytosis (Loke *et al.*, 1982).

Sutton, Mason & Redman (1983) found stellate HLA-DR-positive cells with widespread cytoplasmic processes which they identified as bone marrow-derived immunologically competent cells in the stroma of term chorionic villi. Placental macrophage function changes with advanced gestation; they may acquire the ability to process and present antigen to lymphocytes (Wood & King, 1982).

The purpose of this report was to examine in detail different macrophage and T-lymphocyte populations in human term placenta by using a panel of monoclonal antibodies against macrophage and T-lymphocyte antigens.

MATERIALS AND METHODS

Placentas

Human placentas were collected at the end of the third trimester after 38, 39, 40 weeks of pregnancies either from normal term vaginal deliveries or from caesarean sections. All placental biopsies were cut from chorionic villous mesenchyme (a) from the maternal surface of a central cotyledon to include the decidua, and (b) from the fetal part of the placenta. Specimens were snap-frozen in liquid nitrogen and stored at $-80°$ until sectioning.

Monoclonal antibodies

The murine monoclonal antibodies used, together with their optimal working dilutions, are listed in Table 1.

Correspondence: Professor C. Sorg, Institute of Experimental Dermatology, University of Münster, von Esmarch-Str. 56, D-4400 Münster, FRG.

Table 1. Monoclonal antibodies used in the study

mAb specificity	Reference	Subclass	Dilution
27E10 expressed on young macrophages, appearing in early types of inflammatory processes	Zwadlo *et al.* (1986)*	IgG1	1/40
RM3/1 detects macrophages in down-regulatory stages of inflammatory processes	Zwadlo *et al.* (1987)*	IgG1	1/10
25F9 detects a differentiation antigen preferentially expressed on mature, tissue-fixed macrophages of a late stage of inflammation	Zwadlo *et al.* (1985)*	IgG1	1/40
910D7 reacts with an epitope on the nonpolymorphic part of the bimolecular complex of HLA-DR antigens	Sorg *et al.* (1985)*	IgG2a	1/150
IOT-4 $CD4^+$	Unpublished data†		1/100PAF
IOT-8 $CD8^+$	Unpublished data†		1/150PAF

* Source: Institute of Experimental Dermatology, University of Münster, FRG.
† Source: Immunotech Laboratory, Marseille, France.
PAF, pure ascidic fluid.

Indirect immunoperoxidase labelling

For immunohistology, specimens were rapidly embedded in O.C.T. (Lab Tec Division, Miles, Naperville, IN). Serial cryostat sections (5–7 μm) were cut with a minotome (SLEE, Mainz), fixed in acetone at room temperature for 10 min and air-dried. Then the sections were washed in phosphate-buffered saline (PBS) and incubated in 0·4% sodium acide with 0·1% H_2O_2 in PBS for 7 min in order to inactivate endogenous peroxidases. After washing twice in PBS, the indirect immunoperoxidase labelling was performed. Preincubation was carried out with 50% NGS for 30 min, followed by incubation with 100 μl of the monoclonal mouse anti-human antibodies for each biopsy (Table 1). As a negative control, mouse IgG was included. Monoclonal antibody 910D7, reacting with an epitope on the non-polymorphic part of the bimolecular complex of the HLA-DR antigen (Sorg *et al.*, 1985) was included as positive control.

After washing three times with PBS, the sections were incubated for 30 min at 37° with goat anti-mouse IgG peroxidase conjugate (Dianova, Hamburg) at a dilution of 1/100 in PBS, supplemented with 10% NGS. After three additional washes bound peroxidase was developed in the dark with 3-amino-4-ethylcarbazole (AEC; Sigma, München) at pH 4·9 in 0·1 M acetate buffer plus 0·015% H_2O_2 for 7 min. Counterstaining was performed with Mayer's Hemalum (Merck, Darmstadt).

Double-labelling technique

Cryostat sections of placental biopsies were prepared and preincubated as described above, followed by incubation with 100 μl monoclonal antibody for each section. After washing twice in PBS, the sections were incubated with biotin-conjugated sheep anti-mouse IgG at a dilution of 1/400 in PBS (Amersham-Buchler, Braunschweig) and then washed three times in PBS. This step was followed by incubation with the second monoclonal antibody. Again, sections were washed three times in PBS. Thereafter, they were incubated with alkaline phosphatase-conjugated $F(ab')_2$ goat anti-mouse IgG at a dilution of 1/100 in PBS (Dianova, Hamburg). Each incubation step needs a time period of 30 min. After three additional washes in PBS, bound peroxidase was developed in the dark with 0·1 M 3-amino-4-ethylcarbazole (Sigma) and alkaline phosphatase was developed with fast blue salt (0·5 ng/ml) (Sigma) in naphthol AS-MX phosphate alkaline solution, pH 8·6, 14% v in distilled water, containing 0·2% levamisole.

Evaluation of the immunohistological findings

Macrophage infiltration in representative regions of chorionic villous mesenchyme, decidua and amniochorion was determined semi-quantitatively per microscopic field (magnification ×200).

RESULTS

Distribution of macrophages

The monoclonal antibody 25F9, belonging to the IgG1 subclass, detects a differentiation antigen preferentially expressed on mature, tissue-fixed macrophages of a late stage of inflammation (Zwadlo *et al.*, 1985). In chorionic villous stroma, mononuclear cells reacting with the macrophage antibody 25F9 were demonstrated solely and in small numbers in the fetal villous mesenchyme in 26 of the 36 examined term placentas. They were scattered sparsely. Mainly, they could be identified in the neighbourhood of placental villous blood vessels and—occasionally—in intervillous spaces. In some specimens, the $25F9^+$ cells were large and round, resembling the Hofbauer cells described by other investigators (Castellucci *et al.*, 1980; Fox *et al.*, 1969; Enders & King, 1970) (Fig. 1b), but mostly they were irregularly shaped and located in the neighbouring areas of fibrinoid necrosis.

In decidua basalis and basal plate located on the maternal side of the chorion laeve cytotrophoblast, scattered, irregularly shaped $25F9^+$ cells were consistently noted. The appearance and distribution of the cells were similar to those in the chorionic villous stroma, i.e. the fetal aspect of the placenta.

The monoclonal antibody 27E10 belongs to the IgG1 subclass and detects a differentiation antigen on 20% of peripheral monocytes as well as on inflammatory macrophages in an early stage of inflammation (Zwadlo, Schlegel & Sorg, 1986).

In fetal chorionic villous stroma, this monoclonal antibody shows a quite frequent and distinct reaction pattern; $27E10^+$ macrophages appear either within or adhering to placental villous blood vessels or are located in the tissue. Rarely are they

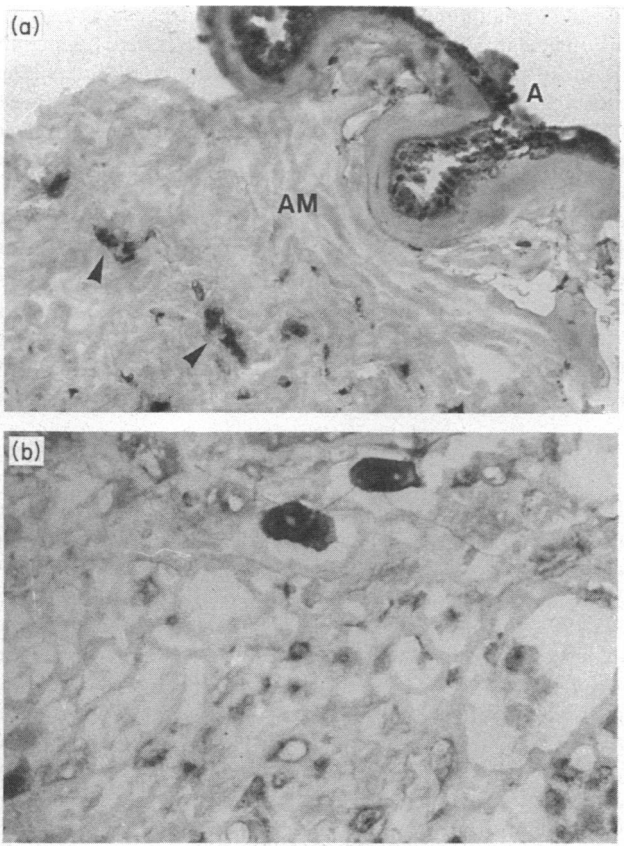

Figure 1. (a) Term amniochorion. Indirect immunoperoxidase staining with monoclonal antibody 25F9 showing irregularly shaped mononuclear cells (arrows) (magnification ×166). (b) Indirect immunoperoxidase staining with monoclonal antibody 25F9, showing two Hofbauer cells within an infarced area of chorionic villous mesenchyme (magnification ×839). A, amniotic epithelium, AM, amniotic mesenchyme.

found in intervillous spaces or at the borderline between syncytiotrophoblast stroma and intervillous spaces.

In decidua basalis and basal plate, i.e. the maternal aspect of the placenta, 27E10$^+$ macrophages are distributed in and around blood vessels as well. Mostly they are found in the immediate vicinity of foci of inflammation and calcification.

Monoclonal antibody RM3/1 belongs to the IgG1 subclass, detecting a surface antigen on 20% of peripheral blood monocytes and a macrophage differentiation antigen associated with the down-regulatory, i.e. healing phase, of the inflammatory process. Dexamethasone strongly enhances the RM3/1 antigen expression *in vitro* (Zwadlo *et al.*, 1987). In placenta, we found this macrophage phenotype to be the major cell population. Large numbers of RM3/1$^+$ macrophages were found within the fetal villous mesenchyme in all placentas examined. RM3/1$^+$ macrophages were detected within the chorionic villi and intervillous spaces and immediately adhering to the cytotrophoblastic shell. They were not found in the neighbourhood of villous blood vessels. RM3/1$^+$ macrophages are of variable morphology, many being irregularly formed whilst others are rounded (Fig. 2). Decidua and basal plate tissue that separates at term, attached to the maternal face of the villous placenta, were found to contain a marked infiltration with RM3/1$^+$ macrophages (Fig. 3).

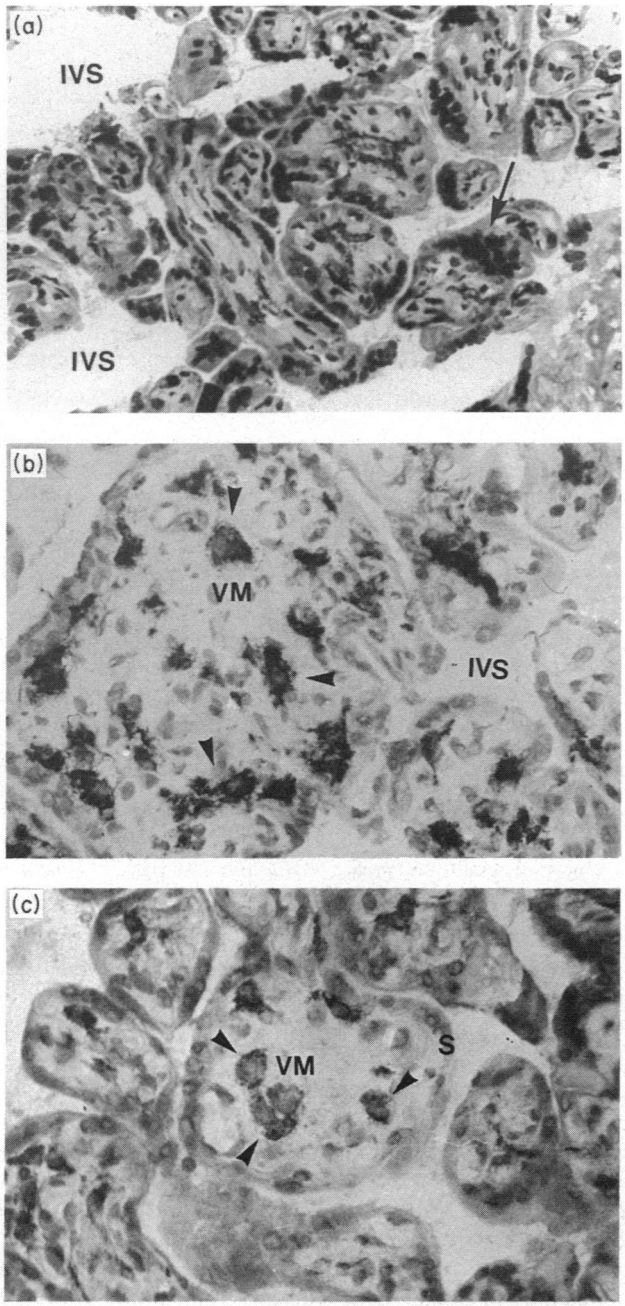

Figure 2. Term fetal chorionic villous tissue. Indirect immunoperoxidase staining with monoclonal antibody RM3/1. (a) Strong positivity of mononuclear cells within villous mesenchyme and the syncytiotrophoblast borderline (arrow) (magnification ×213). (b) Showing stellate shaped mononuclear cells within villous mesenchyme (arrows) (magnification ×416). (c) Showing mononuclear cells within villous mesenchyme. The syncytiotrophoblast does not stain (magnification ×333). IVS, intervillous space; VM, villous mesenchyme; S, syncytiotrophoblast.

Regarding 27E10$^-$, 25F9$^-$, RM3/1$^+$ macrophage phenotypes, their distribution pattern in the amniochorion, present within the mesencymal connective tissue between chorion laeve cytotrophoblast and amniotic epithelium, is similar to that seen in chorionic villous mesenchyme and in decidua. Here too—although to a lesser extent than in decidua—RM3/1$^+$ macro-

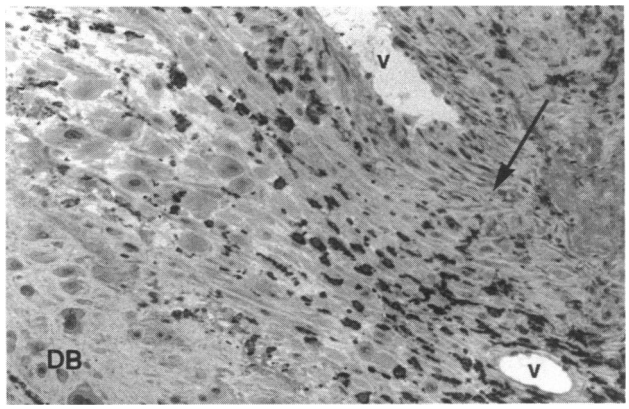

Figure 3. Decidua basalis, i.e. maternal aspect of the placenta. Indirect immunoperoxidase staining using monoclonal antibody RM3/1 showing numerous RM3/1$^+$ macrophages infiltrating the tissue (arrow) (magnification × 104). DB, decidua basalis; V, vessel.

Table 2. Frequency of 25F9$^+$, 27E10$^+$, RM3/1$^+$ macrophages in 36 term placenta biopsies

Biopsy numbers	25F9$^+$*	Biopsy numbers	27E10$^+$	Biopsy numbers	RM3/1$^+$
10/36	0	1/36	0	1/36	0
23/36	+	16/36	+	3/36	+
3/36	++	17/36	++	1/36	++
0/36	+++	12/36	+++	31/36	+++

* Semi-quantitative evaluation of cell numbers per vision field (magnification ×200; see the Material and Methods); 0, virtually no positive cells; +, 5–10 positive cells; ++, more than 10 positive cell; +++, more than 50 positive cells.

phages are the dominating cell population. RM3/1$^+$ macrophages are distributed throughout the tissue and sometimes are concentrated along the edge of the chorionic plate. If present, 25F9$^+$ cells are scattered in small amounts (Fig. 1a), while 27E10$^+$ cells are distributed in and around blood vessels.

The semi-quantitative evaluation of various macrophage populations in term placenta biopsies is summarized in Table 2. More than 50 RM3/1$^+$ macrophages per vision field were found in 31 out of 36 term placentas. More than 50 27E10$^+$ macrophages were found in 12 out of 36 term placentas throughout placental villous core. More than 50 25F9$^+$ macrophages were found in none of the investigated placentas.

In double-staining experiments, it was investigated whether the antigens detected by monoclonal antibodies 25F9, 27E10 and RM3/1 were co-expressed by the same cells. It was found that RM3/1$^+$ and 27E10$^+$ macrophages belong to two different populations, whereas the few 25F9$^+$ macrophages also bore the RM3/1 marker.

Distribution and frequency of CD4$^+$ and CD8$^+$ cells

We also investigated placental biopsies according to their content of CD8$^+$ and CD4$^+$ cells. If present, CD4$^+$ cells were found in small numbers ranging from 1 to 10 positive cells per vision field in 22 out of 36 biopsies. CD4$^+$ cells were found only in those placental biopsies—mainly within the fetal villous mesenchyme—which contained 27E10$^+$ macrophages as well. CD8$^+$ cells were found in greater amounts than CD4$^+$ cells in term placental biopsies. Although 16 biopsies did not contain any CD8$^+$ cells per vision field, in 20 biopsies we found numbers of positive cells ranging from 1 to over 50 per vision field. Furthermore, CD8$^+$ cells were found in intervillous spaces, in the neighbourhood of fetal blood vessels, immigrating into the tissue and also scattered within the amniochorion.

In all investigated placental biopsies we found 25F9$^+$ macrophages and CD8$^+$ cells appearing simultaneously within placental villous core.

DISCUSSION

It has been shown that placental biopsies contain numerous macrophages within the chorionic villous mesenchyme, the decidua and the stroma of the amniochorion (Beer & Sio, 1980; Wilson, Haas & Weaver, 1983; Sutton *et al.*, 1986; Nehemiah *et al.*, 1981; Edwards *et al.*, 1985). Using monoclonal antibodies against different macrophage phenotypes, a more precise evaluation of macrophage heterogeneity in human placenta was possible. In term placenta, decidua and amniochorion maternal and fetal macrophages are in close proximity. Maternal macrophage populations in decidua and basal plate, i.e. the maternal aspect of the placenta, as well as fetal macrophage populations within chorionic villous mesenchyme and amniochorion, have been found to stain positive with our monoclonal anti-macrophage antibodies.

Macrophages stained with the monoclonal antibody 25F9, which detects resident, tissue-fixed macrophages in liver, lung, skin and thymus and cells in late stages of inflammation (Zwadlo *et al.*, 1985), were nearly absent in the placenta. Few 25F9$^+$ cells were found in the amniochorion (Fig. 1a). More frequently than 25F9$^+$ cells, we found macrophages detected by monoclonal antibody 27E10. These are macrophages which mainly appear in acute early stages of inflammatory processes (Zwadlo *et al*, 1986). The significance of the presence of 27E10$^+$ macrophages in placenta is not clear.

In all biopsies we found 27E10$^+$ macrophages and CD4$^+$ cells appearing simultaneously within placental villous core. The simultaneous appearance of 25F9$^+$ macrophages and CD8$^+$ cells was also observed. Since the antigen 27E10 is expressed by activated macrophage phenotypes, which appear at the onset of an inflammatory reaction (Zwadlo *et al.*, 1986), their presence in human term placenta may reflect influx of inflammatory macrophages in response to various infectious stimuli.

The majority of placental macrophages is stained by the monoclonal antibody RM3/1 (Fig. 2). The antibody RM3/1 detects an antigen, which is down-regulated by interferon-gamma, lipopolysaccharide and triphorbolacetate on *in vitro* cultured monocytes, suggesting that the RM3/1 antigen is expressed by a non-inflammatory type of macrophages. This suggestion is supported by the finding that dexamethasone strongly induces the expression of RM3/antigen (Zwadlo *et al.*, 1987).

Kinetic studies of experimental gingivitis indicate that RM3/1$^+$ macrophages increase during the healing phase and

decrease during onset of inflammation (Topoll *et al.*, 1989). Other *in vivo* investigations have shown that RM3/1$^-$ macrophage populations are the predominant cells in liver and heart allografts from patients receiving a high dose corticosteroid medication (Steinhoff *et al.*, submitted). These *in vitro* and *in vivo* characteristics of RM3/1 antigen may also explain the abundance of RM3/1$^+$ cells in placenta, where they may not only phagocytize cellular debris (Bulmer & Johnson, 1984) but also may be involved in the inhibition of specific immune responses (Allison, 1978).

Although macrophages are generally essential for antigen presentation and activation of T cells, they can also suppress the immune response (Kabawat *et al.*, 1985). The dominance of a corticosteroid-induced anti-inflammatory macrophage phenotype within placental villous core and decidua infiltrates suggests localized immunosuppression.

Our observation that decidua is heavily infiltrated by macrophages is in agreement with other reports (Bulmer & Johnson, 1984; Kabawat *et al.*, 1985). The dominance of a corticosteroid-inducible macrophage phenotype, the sparsely presented T lymphocytes, suggests that in human placenta a non-T-cell mediated immunosuppression may occur, probably mediated by functionally and histologically different macrophage populations.

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REFERENCES

ALLISON A.C. (1978) Macrophage activation and nonspecific immunity. In: *International Review of Experimental Pathology* (eds G.W. Richter and M.A. Epstein), Vol. 18, p. 304. Academic Press, New York.

BEER A.E. & SIO J.O. (1980) Maternal immunoregulation and the immunologically privileged status of the trophoblast. In: *Immunological Aspects of Infertility and Fertility Regulation* (eds D.S. Dhindas and G.F.B. Schumacher), p. 143. Elsevier North Holland, Inc., New York.

BILLINGHAM R.E. (1981) Immunobiology of the maternal–fetal relationship. In: *Progress in Clinical and Biological Research, Reproductive Immunology* (eds N. Gleicher), Vol. 70, p. 63. Alan R. Liss, Inc., New York.

BULMER J.N. & JOHNSON P.M. (1984) Macrophage populations in the human placenta and amniochorion. *Clin. exp. Immunol.* **57,** 393.

BULMER J.N. & SUNDERLAND C.A. (1984). Immunohistological characterisation of lymphoid cell populations in the early human placental bed. *Immunology*, **52,** 349.

CASTELLUCCI M., ZACCHEO D. & PESCETTO G. (1980) A three-dimensional study of the normal human placental villous core. *Cell. Tiss. Res.* **210,** 235.

CHAOUAT G. & VOISIN, G.A. (1979) Regulatory T cell subpopulations in pregnancy: evidence for suppressive activity of early phase of MLR. *J. Immunol.* **122,** 1383.

EDWARDS J.A., JONES D.B., EVANS P.R. & SMITH J.L. (1985) Differential expression of HLA class II antigens on human fetal and adult lymphocytes and macrophages. *Immunology*, **55,** 489.

ENDERS A.C. & KING B.F. (1970) The cytology of Hofbauer cells. *Anat. Rec.* **167,** 231.

ENGLEMANN, E.G., MCMICHAEL A.J., BATEY M.E. & MCDEVITT H.O. (1972) A suppressor T cell of the mixed lymphocyte reaction in man specific for the stimulating alloantigen. Evidence that identify HLA-D between suppressor and responder is required for suppression. *J. exp. Med.* **147,** 137.

FOX H. & KHARKONGOR N.F. (1969) Enzyme histochemistry of the Hofbauer cells of the human placenta. *Obstet. Gynecol. Br. Cwlth.* **76,** 918.

JACOBY D.R., OLDING L.B. & OLDSTONE M.B.A. (1984) Immunologic regulation of fetal-maternal balance. *Adv. Immunol.* **35,** 157.

KABAWAT S.E., MOSTOUFI-ZADEH M., DUSCOLL S.G. & BHAN A.K. (1985) Implantation site in normal pregnancy. A study with monoclonal antibodies. *Am. J. Pathol.* **118,** 76.

LALA P.K., CATTERJEE-HASROUNI S., KEARUS M., MONTGOMERY B. & COLAVINCENZO V. (1983) Immunobiology of the feto-maternal interface. *Immun. Rev.* **75,** 87.

LOKE Y.W., EREMIN O., ASHBY I. & DAY S. (1982) Characterization of the phagocytic cells isolated from the human placenta. *RES* **31,** 317.

NEHEMIAH J.L., SCHNITZER J.A., SCHULMAN H. & NOVIKOFF A.B. (1981) Human chorionic trophoblasts, decidual cells, and macrophages: a histochemical and electron microscopic study. *Am. J. Obstet. Gynecol.* **141,** 261.

SORG C. BRÖCKER E.-B., ZWADLO G., REDMANN K., FEIGE U., AX W. & FELLER A.C. (1985) A monoclonal antibody to a formaldehyde-resistant epitope on the nonpolymorphic constant part of the HLA-DR antigen. *Transplantation*, **39,** 90.

STEINHOFF G., WONIGEIT K., SORG C., BEHREND M., MUES B. & PICHLMAYR R. (1989) Patterns of macrophage immigration and differentiation in human liver grafts. *Transplant. Proc.*, submitted.

SUTTON L., GADD M., MASON D.Y. & REDMAN C.W. (1986) Cells bearing class II MHC antigens in the human placenta and amniochorion. *Immunology*, **58,** 23.

SUTTON L., MASON D.Y. & REDMAN C.W. (1983) HLA-DR positive cells in the human placenta. *Immunology*, **49,** 103.

THOMAS I.K. & ERICKSON K.L. (1986) Gestational immunosuppression is mediated by specific Lyt 2$^+$ T cells. *Immunology*, **57,** 201.

TOPOLL H.H., ZWADLO G., LANGE D.E. & SORG C. (1989) Phenotypic dynamics of macrophage subpopulations during human experimental gingivitis. *J. Periodont. Res.* **24,** in press.

WILSON C.B., HAAS J.E. & WEAVER W.M. (1983) Isolation, purification and characteristics of mononuclear phagocyte from human placentas. *J. immunol. Meth.* **56,** 305.

WOOD G.W. & KING C.R. (1982) Trapping antigen-antibody complexes within the human placenta. *Cell. Immunol.* **69,** 347.

ZWADLO G., BRÖCKER E.-B., VON BASSEWITZ D.B., FEIGE U. & SORG C. (1985) A monoclonal antibody to a differentiation antigen present on mature human macrophages and absent from monocytes. *J. Immunol.* **134,** 1487.

ZWADLO G., SCHLEGEL R. & SORG C. (1986) A monoclonal antibody to a subset of human monocytes found only in the peripheral blood and inflammatory tissues. *J. Immunol.* **13,** 512.

ZWADLO G., VOEGELI R., SCHULZE OSTHOFF K. & SORG C. (1987) A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. *Exp. Cell. Biol.* **55,** 295.